

### 53 INVITED HPMA copolymer-TNP-470 (caplostatin) and Avastin show synergistic inhibition of human tumor growth in mice

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Inhibition of angiogenesis, a concept proposed by Judah Folkman in 1971, is one of the most promising approaches for treating human tumors. The formation of capillaries from preexisting blood vessels is now considered to be a key point for tumor growth beyond a critical size of approximately 1 mm<sup>3</sup>. Solid tumors can trigger this complex process by expression of angiogenic factors. Of particular clinical interest is the vascular endothelial growth factor (VEGF); its expression correlates with vessel density and poor prognosis in various tumors. Therapies directed against VEGF or its receptors are showing efficacy in cancer treatment. Recently, this modality has received validation in a large, Phase III clinical trial in metastatic colorectal cancer patients. Monoclonal antibody to VEGF, Avastin, plus chemotherapy resulted in a highly significant longer time to progression and greater survival than chemotherapy alone. Avastin is approved for clinical use in 28 countries.

TNP-470, a broad spectrum angiogenesis inhibitor, has also shown promise in clinical trials, however, doses necessary for tumor regression, showed signs of neurotoxicity. We recently described the synthesis and characterization of a novel non-toxic, water-soluble N-(2-hydroxy-propyl)methacrylamide (HPMA) copolymer-TNP-470 conjugate, now called **caplostatin** [Nature Medicine 10, 255 (2004)]. Conjugation of TNP-470 to HPMA copolymer eliminated its neurotoxicity while retaining its antiangiogenic and anti-tumor activity. Moreover, caplostatin has an improved pharmacokinetic profile, all of which could facilitate its return to clinical trials.

Here we describe the combination of two angiogenesis inhibitors, caplostatin and Avastin with the goal of augmenting the effects of either drug alone. Targeting the endothelial cell, rather than the tumor itself, avoids issues of accessibility to the tumor interior and drug resistance may be less likely. We propose that the antitumor spectrum and efficacy of Avastin can further increase when administered in combination with caplostatin. Our findings demonstrate the high antiendothelial/antitumoral efficacy of the concurrent administration of caplostatin and Avastin *in vitro*. Furthermore, we show the synergistic effect of the combination on 6 different subcutaneous and orthotopic tumor models with a 50% of tumors undergoing complete regression in the COLO-205 human colon carcinoma model. A potential explanation for the favorable combination would be that Avastin inhibits tumor growth by targeting VEGF while VEGF is only one of many angiogenesis stimulators. TNP-470 which has the broadest spectrum of any known antiangiogenic/anti-cancer agent, may target multiple pathways. Our data suggest that combining two non-toxic angiogenesis inhibitors can have increased synergistic anti-tumor effect and no toxicity. The clinical translation of this novel combination is warranted.

### 54 INVITED Regulation and targeting of the hypoxia-inducible factor pathway

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Hypoxia-inducible factor-1 (HIF-1) is a transcriptional complex consisting of HIF-1 $\alpha$  and HIF-1 $\beta$  subunits and is central to regulating genes involved in cell survival and proliferation, vascularisation and metabolic adaptation. HIF-1 activity is dependent on the availability of the  $\alpha$  subunit, which is regulated by cellular oxygen and growth factors. Upon induction, HIF-1 $\alpha$  localises to the nucleus, where it binds to HIF-1 $\beta$  to form the HIF-1 complex. Overexpression of HIF-1 $\alpha$  has been observed in many human cancers and correlates with an aggressive tumour phenotype. Therefore, HIF-1 and components of the HIF-1 pathway are attractive therapeutic targets.

To identify small molecule inhibitors of the HIF-1 pathway, we developed and implemented a cell-based assay that we used recently in a high-throughput screen [Chau et al. *Cancer Research* 2005; 65(11): 4918-28]. We identified two novel hit compounds, CCT001268 and CCT002847 that inhibited HIF-1 activity and HIF-1 $\alpha$  protein induced by the hypoxia mimetic agent, desferoxamine mesylate without significantly inhibiting constitutive luciferase activity in our control cells. CCT001268 but not CCT002847 inhibited HIF-1 activity and HIF-1 $\alpha$  protein induced by hypoxia and significantly inhibited VEGF and Glut-1 expression. Interestingly, both CCT001268 and CCT002847 inhibited HIF-1 $\alpha$  protein induced by insulin-like growth factor-1. Furthermore, we have shown that CCT001268 is a potent inhibitor of VEGF-mediated angiogenesis *in vitro*, has favourable pharmacokinetic properties *in vivo*, and have identified its potential mechanism of action.

HIF-1 $\alpha$  belongs to a growing family of structurally related proteins. HIF-2 $\alpha$ , like HIF-1 $\alpha$  is sensitive to multiple stimuli and can be constitutively expressed. However, the relative contribution of HIF-1 $\alpha$  and HIF-2 $\alpha$  to HIF-regulated target genes under different conditions is unclear. Using siRNA techniques to knockdown HIF-1 $\alpha$  and/or HIF-2 $\alpha$  expression, we found that HIF-1 $\alpha$  primarily regulates transcriptional activation of VEGF in response to hypoxia and IGF-1 as compared with HIF-2 $\alpha$  in MCF-7 cells. In renal carcinoma cells that constitutively express both HIF-1 $\alpha$  and HIF-2 $\alpha$  due to loss of von Hippel-Lindau (pVHL) E3 ligase function, we found that high basal VEGF, GLUT-1, uPAR and PAI-1 expression was predominantly dependent on HIF-2 $\alpha$ . Furthermore, we found a reciprocal relationship between HIF-1 $\alpha$  and HIF-2 $\alpha$  expression in these cells. Our observations suggest that HIF-targeted gene expression is stimulus and cell-type dependent, and that small molecule inhibitors of the HIF pathway ideally should target both HIF-1 $\alpha$  and HIF-2 $\alpha$  expression. Accordingly, we found that CCT001268 blocked constitutive HIF-1 $\alpha$ , HIF-2 $\alpha$  and VEGF expression in renal carcinoma cells. Our data have important implications for how we target the HIF pathway therapeutically.

### 55 INVITED The MET oncogene drives a genetic program linking cancer to haemostasis

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The close relationship between activation of blood coagulation and cancer is an old enigma. In 1865 *migrans trombophlebitis* (a condition of the blood that predisposes it to spontaneous coagulation) was described as forewarning of occult malignancy (Trousseau's sign). This pioneer observation emphasized the existence of haemostasis disorders associated with cancer onset, since then extensively reported in clinical and epidemiological studies, but so far orphan of a mechanistic explanation. Here we report a mouse model of sporadic tumorigenesis based on genetic manipulation of somatic cells. Targeting the activated *MET* oncogene to adult liver caused slowly progressing hepatocarcinogenesis. Surprisingly, this was preceded and accompanied by a syndrome manifesting first with blood hypercoagulation (venous thromboses), and then evolving towards fatal internal haemorrhages. The pathogenesis of this syndrome is driven by the transcriptional response to the oncogene, including prominent up-regulation of plasminogen activator inhibitor type 1 (PAI-1) and cyclooxygenase-2 (COX-2) genes. *In vivo* analysis showed that both proteins support the thrombohaemorrhagic phenotype, thus providing direct genetic evidence for the long-sought link between oncogene activation and haemostasis.

### 56 EACR Award The European Association for Cancer Research (EACR) Young Cancer Research Award – Molecular regulation of an invasion-related molecule: options for tumor staging and clinical strategies

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The urokinase-receptor (u-PAR) promotes the invasive and metastatic phenotype and has been shown to be associated with early relapse and poor prognosis in numerous types of cancers. In addition, we have shown that expression of the u-PAR on minimal residual tumor cells in the bone marrow of patients with gastric cancer indicates a metastatically relevant phenotype of these early disseminated tumor cells, and that the detection of u-PAR on these cells at the time of primary surgery can predict the development of a clinically relevant minimal residual disease component, early relapse, and poor prognosis. From our and other studies we know that high u-PAR gene expression in carcinoma cells is largely due to the transcriptional regulation of the gene. We have characterized two cis-elements (-152/-135, bound with an AP-2-like protein, Sp1, and Sp3; -190/-171, bound with AP-1-transcription factors) of the u-PAR promoter which are decisive for diverse means of u-PAR-gene expression in highly invasive colon cancer cells: The AP-2/Sp1 element is mediating constitutive and PMA-induced gene expression, and, more importantly, induction of u-PAR-mediated proteolysis brought about by c-Src, and suppression brought about by the newly characterized tumor suppressor Pdc4. The AP-1-motif is mediating constitutive gene expression, as well as induction of u-PAR-mediated proteolysis brought about by K-Ras. U-PAR-mediated proteolysis could be inhibited by specific Src- and Ras-inhibition, as well as transcriptionally by dominant-negative AP-2.

The lecture will focus on the differential binding of transcription factors to both u-PAR promoter elements *in vivo*, having been investigated in a large series of resected tumor and normal tissue of colorectal and gastric cancer patients. We will demonstrate that, depending on the transcription

factor and cis-element, patient subgroups of different size can be selected in which transactivation via these promoter elements might be tumor-tissue-specific, suggesting subgroups for tumor-selective targeting. Also, the lecture will outline that different u-PAR-promoter motifs may be of different tumor-specificity in vivo. We will also suggest patient subgroups in which a synergistic regulation of u-PAR gene expression in resected tissues via both promoter elements can be postulated. Finally, potential conclusions for a more target-oriented patient selection and therapy out of transcriptional and oncogenic regulators of the uPA-R gene will be discussed. The lecture will in part contain new, non-published data.

### Keynote Lecture

#### Oncogenes and the future of cancer therapy

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INVITED

##### Oncogenes and the future of cancer therapy

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Over the past thirty five years, several methods have been used to determine whether dominantly acting oncogenes that have been implicated in the initiation of tumorigenesis are also required to maintain the transformed state and the viability of cancer cells. These approaches include (i) the use of conditional [temperature-sensitive] mutants of retroviral oncogenes and, more recently, small inhibitory RNA's that target oncogenic mRNA's in an allele-specific fashion; (ii) the generation of transgenic mice in which oncogenes or their protein products can be activated and then deactivated (e.g. by the use of antibiotic-dependent systems for gene induction or hormone-sensitive fusion proteins); and (iii) the introduction of protein-specific inhibitory agents, mainly small molecules and antibodies, that can be examined for therapeutic effects in patients, as well as in experimental animals and cell culture.

A striking and encouraging theme has emerged from these various strategies: the idea that, in general, cancer cells seem to be dependent on one or a few activated oncogenes for maintenance of their oncogenic status and even for cell viability. This implies that, for any cancer, it might be possible to control and even eliminate tumor cells with one agent or a small combination of targeted drugs or antibodies, despite extensive genetic changes in the cancer cell. To accomplish this, it will be necessary to catalog in more detail the genotypes of the many kinds of human cancer and to discover more therapies targeted specifically against more of the many genes and proteins that have been implicated in carcinogenesis by gain-of-function mutations.

I will discuss recent studies of lung adenocarcinomas arising spontaneously in human patients or induced in mice with oncogenes belonging to the Ras and epidermal growth factor receptor families. Information about oncogene-dependence of tumors in the mouse models, about the importance of genotypes in the selection of drugs to treat human lung cancer, and about the role of secondary mutations in acquired resistance to inhibitors of protein-tyrosine kinases will be used to advance the case that most or all cancers will ultimately become highly controllable disorders.

### Young Oncologists session

#### Life after cancer treatment

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INVITED

##### Persistent fatigue in cancer survivors: biological basis and clinical management

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Fatigue is one of the most commonly reported symptoms for patients receiving cancer treatment and this may persist beyond the acute phase of treatment among cancer survivors. Persistent fatigue is reported by as many as 30% of breast cancer survivors, and is also a frequent complaint for other survivors (e.g. Hodgkin's disease). Persistent fatigue is often associated with pain and depressive symptoms; however, it is uncertain if these associations represent cause or effect. In our laboratory, we have been studying some of the biological and psychological mechanisms of fatigue in cancer survivors, and these will be explored in this presentation. Strategies to address fatigue in the clinic are multifactorial and depend on the etiology of the symptom.

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INVITED

##### Management of long-term sequelae in patients with Hodgkin's disease

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The survival of patients treated for Hodgkin's lymphoma (HL) has improved dramatically as a result of the development of multiagent chemotherapy, more accurate radiotherapy (RT), and enhanced possibilities to treat complications during and after treatment. Nevertheless, it is evident that the average survival for HL patients does not return to normal after treatment when compared with age-matched general population. The two major causes of mortality excluding HL in the long-term survivors are secondary malignancies and cardiovascular disease (CVD). When compared with the general population, the leukaemia risk in HL patients is 10 to 80 higher. The risk period for leukaemia is predominantly within the first 10 years. Young females who receive thoracic RT are at high risk of developing breast cancer. There was no increased risk for patients >30 years but the risk was 56 times greater for patients ≤19 years old. HL patients have also an increased risk of developing lung cancer, sarcomas, melanomas and thyroid cancer. With respect to CVD, the relative risk of death from cardiac disease has been estimated to increase 3.1 times with the highest risk group being that of young patients treated with mediastinal RT. Other long-term complications include infectious episodes, pulmonary fibrosis, osteonecrosis, hypothyroidism and gonadal dysfunction. Finally, fatigue levels of patients with HL are high, even years after treatment. From a management point of view, it is important to pay attention to possible signs of malignancies – especially of the digestive and respiratory tract and especially in patients treated at young ages. For women who have been irradiated before age 30, screening is urged because of the highly elevated risk of developing breast cancer. Pneumococcal vaccination and instructions on the use of antibiotics are recommended after splenectomy. Patients treated for HL should be advised to refrain from smoking, because smoking acts synergistically with RT in the development of lung cancer and, potentially, CVD. Timely intervention in other risk factors of CVD may help to reduce its high absolute excess risk in survivors of HL. Finally, the development of risk-adapted therapeutic strategies based on new developments in diagnostic testing and enhanced understanding of the basic pathogenic mechanisms of HL, including molecular profiling, may also assist in the identification of specific prognostic groups that might receive different therapies.

60 Abstract not received